

Protocol

DNA Transfection by Electroporation

Priti Kumar, Arvindhan Nagarajan, and Pradeep D. Uchil

Electroporation, which uses pulsed electrical fields, can be used to introduce DNA into a variety of animal cells, plant cells, and bacteria. Electroporation works well with cell lines that are refractory to other transfection techniques, such as lipofection and calcium phosphate–DNA coprecipitation. But, as with other transfection methods, the optimal conditions for electroporating DNA into untested cell lines must be determined empirically. Several different electroporation instruments are available commercially, and manufacturers supply detailed protocols for their use with specific cell types and guidelines for optimization with others. This method describes the conditions for electroporating mammalian cell lines using the Gene Pulser Xcell Electroporation System (Bio-Rad).

MATERIALS

It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous materials used in this protocol.

RECIPES: Please see the end of this protocol for recipes indicated by <R>. Additional recipes can be found online at <http://cshprotocols.cshlp.org/site/recipes>.



Reagents

Carrier DNA (10 mg/mL; e.g., sonicated salmon sperm DNA) (optional)

Cell culture growth medium (complete and [optional] selective)

Electroporation buffer

The electroporation buffer needs to be optimized based on the cell type or on the manufacturer's recommendations. Any of the following buffers could be used: phosphate-buffered saline, HEPES-buffered saline (HBS), phosphate-buffered sucrose, and HEPES-buffered sucrose. For all preset protocols programmed in the Gene Pulser Xcell System, the manufacturer recommends the use of Opti-MEM or growth media without serum as electroporation buffer.

Exponentially growing cell cultures

For best results, passage cells 1–2 d before electroporation, and harvest cells at a density of 60%–80% confluency on the day of electroporation.

Linearized or circular plasmid DNA (1–5 µg/µL in sterile deionized H₂O)

Phosphate-buffered saline (PBS) <R>

Trypsin

From the Molecular Cloning collection, edited by Michael R. Green and Joseph Sambrook.

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Equipment

Electroporation cuvettes

Electroporation instrument

This protocol presumes the use of the Gene Pulser Xcell System with ShockPod chamber for eukaryotic cells (Bio-Rad; catalog no. 165-2661; operational at 100–240 V).

Hemocytometer

Tissue culture dish or multiwell plate

Tissue culture flasks (75 cm²)

METHOD

1. Prepare cells for electroporation.

For Adherent Cells

- i. One day before electroporation, use trypsin to release adherent cells, and transfer the cells into 75-cm² flasks with fresh growth medium at a density sufficient to obtain 50%–70% confluence on the day of electroporation. (For most cell lines, this translates to $\sim 1 \times 10^5$ to 10×10^5 cells per electroporation.)
- ii. On the day of the experiment, aspirate the growth medium, rinse cells with PBS, and use trypsin to release adherent cells. Remove an aliquot of trypsinized cell suspension, and, using a hemocytometer, count the cells to determine the cell density. Centrifuge the cell suspension at 500g for 5 min at room temperature.
- iii. Resuspend the cell pellet in the appropriate electroporation buffer at a density of 1×10^6 to 5×10^6 cells/mL. Gently pipette cells to obtain a single suspension.

For Suspension Cells

- i. One day before electroporation, dilute the cells into fresh growth medium in a 75-cm² flask to obtain a mid-log phase of growth on the day of electroporation confluence ($\sim 0.5 \times 10^6$ to 4×10^6 cells/mL). Count cells and collect them by centrifugation as described above.
- ii. Resuspend the cell pellet at a density of 1×10^6 to 5×10^6 cells/mL in the appropriate electroporation buffer. Gently pipette cells up and down to obtain a homogeneous suspension.

Electroporation

2. Set the parameters on the electroporation instrument. Depending on the cell type, choose either a preset protocol or an optimization protocol. For an exponential protocol (i.e., exponential decay pulse), a typical capacitance value is 1050 μ F, and the voltage ranges from 200 to 350 V. Generally a starting voltage of 260 V with 50-V increments works for most cells. Use an infinite internal resistance value.
3. Add 10–50 μ g of plasmid DNA to the electroporation cuvette. If needed, carrier DNA (e.g., salmon sperm DNA) may be added to bring the total amount of DNA to 120 μ g.
4. Add cells to the cuvette, and gently mix the cells and DNA by pipetting up and down. For suggestions on cell concentrations and volumes, see Table 1.

Do not introduce air bubbles into the suspension during the mixing step.

5. Place the cuvette into the electroporator, close the lid, and apply one electric pulse.
Record the actual pulse time and time constant for each cuvette to facilitate comparisons between experiments.



TABLE 1. Recommendations for electroporation using the Bio-Rad ShockPod

Cuvette (cm)	Cell concentration (cells/mL)	Cell volume (µL)	Growth conditions following electroporation
0.2 ^a	1×10^6	100	Forty-eight-well plate with 0.5 mL of growth medium
0.2	5×10^6	200	Six-well plate with 2 mL of growth medium
0.4	2.5×10^6	400	Six-well plate with 2 mL growth medium

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^aNot recommended for eukaryotic cells.

6. Immediately add 0.5 mL of growth medium to the cuvette, and transfer the electroporated cells to an appropriately sized tissue culture dish or multiwell plate. If desired, rinse out 0.2- or 0.4-cm cuvettes with growth medium, and add the wash to the cells on the plate or dish.
7. Repeat Steps 5 and 6 for each sample and each voltage increment. Record the actual pulse time and time constant for each cuvette to facilitate comparisons between experiments. For mammalian cells, conditions that result in pulse times or time constants of 10–40 msec at field strengths of 400–900 V/cm are optimal. Rock the plate gently to distribute the cells evenly throughout the well or dish.
8. Transfer the dish or plate to a humidified incubator at 37°C with an atmosphere of 5%–7% CO₂. Incubate the cells for 6–24 h.
9. Determine cell viability using any of the methods outlined in Protocol: Analysis of Cell Viability by the alamarBlue Assay (Kumar et al. 2018a), Protocol: Analysis of Cell Viability by the Lactate Dehydrogenase Assay (Kumar et al. 2018b), or Protocol: Analysis of Cell Viability by the MTT Assay (Kumar et al. 2018c).
10. To isolate stable transfectants, follow Step 13 of Protocol: DNA Transfection Mediated by Cationic Lipid Reagents (Kumar et al. 2019a).
11. For transient expression, examine the cells 24–96 h after electroporation using one of the assays described in Step 12 of Protocol: DNA Transfection Mediated by Cationic Lipid Reagents (Kumar et al. 2019a).

DISCUSSION

When working with a new experimental system, begin with an optimization protocol using incremental voltage steps to determine the best electroporation conditions. Optimization can be greatly facilitated by using an electroporator that can handle multiwell electroporation plates.

The efficiency of transfection by electroporation is influenced by the following factors.

- *Strength of the applied electric field.* At low voltage, the plasma membranes of cultured cells are not sufficiently altered to allow passage of DNA molecules; at higher voltage, the cells are irreversibly damaged. For most lines of mammalian cells, the maximal level of transient expression is reached when voltages between 250 and 500 V/cm are applied. Typically, between 20% and 50% of the cells survive this treatment (as measured by exclusion of Trypan Blue) (Patterson 1979; Baum et al. 1994).
- *Duration of the electric pulse.* Usually, a single electric pulse is applied to cells. The duration, field shape, and strength of the pulse are determined by the capacitance of the power supply and the dimensions of the cuvette. Most electroporation devices grant the investigator control over the characteristics of the pulse. The optimal length of the electric pulse required for electroporation is 20–100 msec. The electroporator can also offer the choice of varying the electrical pulse shape between exponential decay and square wave (for pulse wave characteristics, see Introduction: Electroporation [Kumar et al. 2019b]).

- *Temperature.* Some investigators have reported that maximal levels of transient expression are obtained when the cells are maintained at room temperature during electroporation (Chu et al. 1987). Others have obtained better results with cells maintained at 0°C (Reiss et al. 1986). These differences may be cell type-specific or dependent on the amount of heat generated during electroporation when large electrical voltages (>1000 V/cm) and extended electric pulses (>100 msec) tend to produce more heat. The efficiency of transient expression is increased if the cells are incubated for 1–2 min in the electroporation chamber after exposure to the electric pulse (Rabussay et al. 1987).
- *Conformation and concentration of DNA.* Although both linear and circular DNAs can be transfected by electroporation, higher levels of both transient expression and stable transfection are obtained when linear DNA is used (Neumann et al. 1982; Potter et al. 1984; Toneguzzo et al. 1986). Effective transfection has been obtained with concentrations of DNA ranging from 1 to 40 µg/mL. The purity of the plasmid is also a prominent factor that contributes to the efficiency of electroporation. For most practical purposes, plasmid DNA purified by the many commercial kits (preferably those that also use a step to remove bacterial endotoxins) yields satisfactory results.
- *Ionic composition of the medium.* The efficiency of transfection is severalfold higher when the cells are suspended in buffered salt solutions (e.g., HEPES-buffered saline) rather than in buffered solutions containing nonionic substances such as mannitol or sucrose (Rabussay et al. 1987). Some companies also market proprietary buffers recommended for use with any electroporator for any nucleic acid and mammalian cell line, for example, the Gene Pulser Electroporation Buffer (Bio-Rad), or Ingenio Electroporation Solution (Mirus Bio).
- *Cell physiology.* The best transfections are achieved with actively growing, healthy cells that are free of contamination and preferably of low passage number.

For examples of electroporation in animal cells, see Neumann et al. (1982), Wong and Neumann (1982), Potter et al. (1984), Sugden et al. (1985), Toneguzzo et al. (1986), and Tur-Kaspa et al. (1986). For examples of electroporation in plant cells, see Fromm et al. (1985, 1986) and Ecker and Davis (1986).

For further information on the history, mechanism, and optimization of electroporation, see Introduction: Electroporation (Kumar et al. 2019b). For a description of an electroporation technique that transfers DNA directly into the nucleus, see Box 1.

BOX 1. DNA TRANSFECTION BY NUCLEOFECTON

“Nucleofection” is a specialized form of electroporation that delivers nucleic acids through the nuclear membrane and directly into the nucleus. Because DNA or RNA is transferred directly into the nucleus, cell division is not required for the incorporation of nucleic acid into the cell. Thus, nucleofection permits comparatively efficient transfection of nondividing primary cells such as neurons, as well as difficult-to-transfect cell lines. Unlike other prevalent transfection methods, the same conditions are used for the nucleofection of any nucleic acid; however, the electrical parameters and buffer solutions are specific to the cell type being transfected.

The proprietary electrical conditions are preloaded in the nucleofection device, and proprietary cell-specific solutions are required. As with other transfection technologies, the cell conditions (confluence, passage number, etc.), the amount and quality of DNA, and the manner in which cells are handled all influence transfection efficiency. Protocols that have been optimized for more than 500 cell types are available from the manufacturer of the nucleofection instruments and reagents (see <http://www.lonzabio.com/resources/product-instructions/protocols/>). In addition, a cell line optimization kit and optimization kits for primary cells are available to standardize transfection according to one’s experimental setup.

RECIPE

Phosphate-Buffered Saline (PBS)

Reagent	Amount to add (for 1× solution)	Final concentration (1×)	Amount to add (for 10× stock)	Final concentration (10×)
NaCl	8 g	137 mM	80 g	1.37 M
KCl	0.2 g	2.7 mM	2 g	27 mM
Na ₂ HPO ₄	1.44 g	10 mM	14.4 g	100 mM
KH ₂ PO ₄	0.24 g	1.8 mM	2.4 g	18 mM
If necessary, PBS may be supplemented with the following:				
CaCl ₂ •2H ₂ O	0.133 g	1 mM	1.33 g	10 mM
MgCl ₂ •6H ₂ O	0.10 g	0.5 mM	1.0 g	5 mM

PBS can be made as a 1× solution or as a 10× stock. To prepare 1 L of either 1× or 10× PBS, dissolve the reagents listed above in 800 mL of H₂O. Adjust the pH to 7.4 (or 7.2, if required) with HCl, and then add H₂O to 1 L. Dispense the solution into aliquots and sterilize them by autoclaving for 20 min at 15 psi (1.05 kg/cm²) on liquid cycle or by filter sterilization. Store PBS at room temperature.

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